PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

**(PCT Article 36 and Rule 70)**

| Applicant's or agent's file reference<br>KENRYAN | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/US02/32078 | International filing date *(day/month/year)*<br>08 October 2002 (08.10.2002) | Priority date *(day/month/year)* |
| International Patent Classification (IPC) or national classification and IPC<br>IPC(7): A23K 1/00 and US Cl.: 426/52 | | |
| Applicant<br>ROBINSON, LEANNE G. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 40 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 26 September 2003 (26.09.2003) | 19 March 2004 (19.03.2004) |
| Name and mailing address of the IPEA/US<br>Mail Stop PCT, Attn: IPEA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (703) 305-3230 | Authorized officer<br>Sandra Saucier Valerie Bell-Harris for<br>Telephone No. 571/272-1600 |

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US02/32078

## I. Basis of the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed.

☒ the description:
pages NONE as originally filed
pages NONE, filed with the demand
pages 1-34, filed with the letter of 05 February 2004 (05.02.2004)

☒ the claims:
pages NONE, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages 36-40, filed with the letter of 05 February 2004 (05.02.2004)

☒ the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages 1/1, filed with the letter of 05 February 2004 (05.02.2004).

☒ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US02/32078

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. STATEMENT

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 2, 6, 7, 11, 15-17, 19, 21 | YES |
| | Claims | 1, 3-5, 8-10, 12-14, 18, 20, 22 | NO |
| Inventive Step (IS) | Claims | 2, 6, 7, 11, 15-17, 19, 21 | YES |
| | Claims | 1, 3-5, 8-10, 12-14 ,18, 20, 22 | NO |
| Industrial Applicability (IA) | Claims | 1-22 | YES |
| | Claims | NONE | NO |

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

**V. 2. Citations and Explanations:**
Claims 1, 3-5, 8-10, 12-14, 18, 20 and 22 lack novelty under PCT Article 33(2) as being anticipated by US 5,432,074.

The claims are directed to a method of adding a composition comprising fibrolytic enzymes such as cellulase, xylanase, hemi-cellulase, having a ratio of 1 unit of enzyme activity to 10 to the fifth colony-forming units to cattle feed and feeding the mixture to cattle.

US 5,432,074 disclose a cattle feed additive comprising 1,4-xyanase, 1,3-xylosidase and lactic acid bacteria and a method of adding the composition to cattle feed.

Although the reference is silent with regard to the ratio of enzyme activity/CFU, the composition was formulated to ensure the production of at least 2% of fermentable sugars by wet weight and 10 to the fifth bacteria per gram from maize. In the absence of evidence to the contrary, this is presumed to be the same ratio as claimed.

Claims 1, 3, 4, 20 and 22 lack novelty under PCT Article 33(2) as being anticipated by US 6,326,037.

US 6,326,037 disclose a cattle feed additive composition comprising L. buchneri 4.87% and glucanse, xylanase and galactomannanase 80.09% and a method of adding it to cattle feed and feeding cattle.

Although the reference is silent with regard to the ratio of enzyme activity/CFU, the disclosure gives the weight ratio of the composition. In the absence of evidence to the contrary, this is presumed to be the same ratio as claimed.

Claims 1, 3-5, 8-10, 12-14, 18, 20, 22 lack an inventive step under PCT Article 33(3) as being obvious over US 5,053,233.

US 5,053,233 disclose a cattle feed additive comprising: a lactic acid bacteria, L. plantarum, sufficient to yield from $10^5$ to $10^7$ CFU/g feed and a fibrolytic enzyme such as cellulase, hemi-cellulase 150ml/t feed and a method of addition to cattle feed and feeding the mixture to cattle.

While the reference is silent with regard to the ratio of units of enzyme activity/CFU, in the absence of evidence to the contrary, the ratio of amounts of enzyme/bacteria is presumed to be the same as claimed.

**Supplemental Box**
(To be used when the space in any of the preceding boxes is not sufficient)

Applicants arguments have been carefully considered and amendments to the claims which exclude a step of ensilaging have been found to be free of the previously cited art. Arguments regarding neccessary amounts fed and the results obtained are not persuasive because the rejected claims may encompass a step of ensiling. Since the method AS CLAIMED may encompass the method disclosed in the prior art, the results such as weight gain, would reasonably be expected to be the same in the absence of evidence to the contrary.

# Substitute for Animal Protein in Cattle Feed

## Technical Field

The field of the present invention is cattle feed additives, which improve feed nitrogen utilization, and eliminate the need for animal protein supplements, which animal protein supplements can cause disease.

## Background Art

U.S. Patent 5,780,288 Rohwer (1998) discloses at Col. 1, line 15 to Col. 2, line 20, "Creutzfeldt-Jakob Disease (CJD) is a rare neurological disease found in humans, first described in the 1920s and found worldwide. It is usually manifested in late middle-age with progressive dementia and is usually fatal within six months. It is characterized by spongiform changes in the brain, but this can only readily be diagnosed at post mortem. The identification in 1996 of at least 10 cases of CJD in Britain which seem to represent a new variant caused concern that these cases could be linked to exposure to bovine spongiform encephalopathy (BSE), or "Mad Cow Syndrome," which has infected some 160,000 cows in Britain. The distinct variant in these 10 cases occurred in people aged under 42 with dates of onset of illness in the last two years. This variant has not been previously recognized and is characterized by behavioral change, ataxia, progressive cognitive impairment and a tendency to a prolonged duration of illness. In April 1996, Dr. Stanley B. Prusiner of the University of California, San Francisco, presented scientific evidence that he believes indicates a link between CJD and BSE.

In 1988, as a result of earlier concerns about the possible transmission of BSE to humans, the UK adopted control measures which included: 1) destroying cattle clinically diagnosed on the farm, 2) prohibiting feeding cattle and other ruminants material containing animal protein derived from ruminants and 3) destroying carcasses of cattle infected with BSE. The public health threat of the 10 new cases was deemed great enough that the European Union (EU) has imposed further precautionary measures which include: 1) a ban on the international sale of all meat, offal, semen, embryos, and other products of British

cattle, 2) a requirement that carcasses from cattle aged over 30 months must be destroyed, and 3) a prohibition on the use of mammalian meat and bone meal in feed for all farm animals.

The health panic triggered by the evidence that the fatal CJD might be caused by eating beef has fast become a significant economic issue. The cost to the UK and EU alone of destroying cattle which are aged over 30 months is predicted to be approximately $10 billion if the cattle are killed at a rate of 15,000 per week over the next six years.

There have been no reported cases of BSE in the United States; nonetheless, as a preventative measure, a prohibition of imported ruminants from the U.K. was implemented in July 1989. Scrapie and other forms of spongiform encephalopathy are present in the United States, however, causing an intense interest in BSE. Presently, a voluntary practice against feeding ruminant byproducts to ruminants exists in the U.S. There is ongoing discussion among governmental regulatory agencies on whether to impose an official ban on such feeding practices. A related issue of concern in the U.S. is that a transmissible form of spongiform encephalopathy found in ranched mink, Transmissible Mink Encephalopathy (TME), has been primarily attributed to feeding the mink-scrapie-infected sheep and goat carcasses. Cattle carcasses, which are also part of the ranched mink diet, are now a suspected source of TME (Bolis & Gibbs J. Amer. Vet. Med. Assoc., 1990).

BSE is believed to be caused by a biological agent called a prion protein. Prions are unique to the world of biology because they are able to replicate without the benefit of any nucleic acid (e.g., DNA or RNA). Nucleic acids are used by everything from viruses to bacteria to humans to store genetic information. This genetic information is used by organisms to build specific proteins. Proteins, in turn, do the work of the cell. Normally, proteins do not have the ability to vary genetic information. What makes prions very unusual is that they seem to be made exclusively of proteins. Since prions are able to propagate themselves, it is believed that the prion proteins are able to carry genetic information. The

"WAUSAU, Wis., July 31 - The deaths of three outdoorsmen from brain-destroying illnesses are under investigation by medical experts who want to know whether chronic wasting disease has crossed from animals into humans, just as mad cow disease did in Europe."

United States Patent 5,093,121 of Kvanta, et al. March 3, 1992 entitled "Method for increasing the protein contents of milk in ruminants" discloses at the Abstract, "The invention relates to a method of increasing the protein contents of milk in milk producing animals by introducing into the animal a culture of one or more non-pathogenic lactic acid producing live bacteria in admixture with a carrier. The invention also relates to a preparation containing said lactic acid producing bacteria in admixture with a carrier facilitating the optimal growth of the bacteria in the stomach-intestine system of the animal. The invention also deals with the use of non-pathogenic lactic acid producing live bacteria for increasing the protein contents of milk in milk producing animals".

United States Patent: 5,529,793 of Garner, et al., June 25, 1996, entitled, "Compositions for Improving the Utilization of Feedstuffs by Ruminants," discloses at the abstract, "A composition of a mixture of a lactic acid producing bacteria culture and a lactate utilizing bacteria culture admixed with a dry formulation or an animal feedlot diet for improving the utilization of feedstuffs by a ruminant. The composition may be used on a continual basis to increase meat or milk production, or used during the transition from a roughage diet to a feedlot diet to prevent or minimize acidosis. The preferred embodiment utilizes *Lactobacillus acidophilus* as its lactic acid producing bacteria culture and Propionibacterium P-5 as its lactate utilizing bacteria culture. The composition is in a dry powder form for storage at ambient temperatures for long durations."

U.S. Patent 5,662,901 Tobey et al (1997) discloses at the Abstract, "The invention comprises two grain conditioners. The first grain conditioner, which is suitable for all grains, comprises a pectinase, a protease, a beta-glucanase and an amylase. The second grain conditioner, which is designed for use on easier-to-digest grains, comprises a pectinase, a beta-glucanase, an amylase and a hemicellulase." At col.1, lines 58 to 60 Tobey et al

disclose, "The addition of enzymes to animal feeds is also known to increase feed utilization efficiency and weight gain."

U.S. Patent 5,720,971, Enzyme Additive for Ruminant Feeds, Beauchemin et al (1998) discloses at the abstract "--The fibrolytic enzyme supplements consist of mixtures of cellulase and xylanase is certain preferred ratios----. The cellulase and xylanase are dissolved in a buffer and sprayed onto dry legume forages or grain feeds. The feed material is then incubated for at least three hours to allow the enzymes to be absorbed into and adhere to the feed material."

Beauchemin also discloses, "Direct addition of fibrolytic enzymes to the ruminal environment is also unlikely to be of benefit as the rumen contains bacteria, fungi and protozoa which produce the most active cellulase and xylanase known to exist in any environment (Gilbert 1992)."

In an article entitled Fibrolytic enzymes for beef and dairy cows, David Hutcheson PhD., Animal Agricultural Consulting, Inc. PO Box 50367 Amarillo, TX 79159 discloses, "Fibrolytic enzymes increases dry matter digestibility, neutral detergent fiber digestion, organic matter, cellulose, hemicellulose and increase ruminal rates of microbial protein."

U.S. Patent 6,221,381 B1 Shelford (2001) discloses at the Abstract, "Methods and compositions are provided for enhancing feed utilization efficiency in a ruminant animal by adding to the feed a sufficient amount of a nonionic surfactant to enhance the utilization of the feed by the animal.------A digestion enhancing enzyme and lactic acid bacteria inoculum may also be added to the feed." At col. 7, first paragraph, Shelford discloses "In addition to feed and a nonionic surfactant, the compositions of the invention may further comprise one or more additional agents that enhance the ruminant digestive processes. Such agents include, for example, pyrodoxal 5-phosphate, fumaric acid and its salts, sorbic acid and its salts, parabenzoic acid esters, benzoic acid, polydimethyl siloxane-polyethers, unsaturated alcohols, bentonite, proteolytic and/or carbohydrase enzymes, such as glycanase, hemicellase, cellulase, pectinase, xylanase and amylase, and lactic acid bacteria inoculants, such as those

At the end of the growth, the organisms were mixed together, forming a combined liquid culture. Ten gallons of the combined liquid culture was thoroughly mixed with the following ingredients to form a doughy mass: 1.0 pounds of lecithin, 0.1 pounds of sodium propionate, 2.0 pounds calcium carbonate, 2.0 pounds of multi-enzymes, 0.10 pounds of yucca schidigera extract (range 0.01 to 1 pound), 40 pounds of sodium bentonite (range 30 to 60 pounds), 20 pounds of rice flour (range 10 to 30 pounds), and 20 pounds of wheat flour. The pH of the mix was adjusted to 6.5 to 7.5 using sodium hydroxide or sodium bisulfate. The micro-prep was extruded in the form of small pellets. The extruded micro-prep was dried and milled to the consistency of the feed." At claim 6, the enzyme was disclosed as follows, "6. The media system of claim 5, wherein said pellet further comprises an enzyme selected from the group consisting of protease, lipase, amylase, cellulase, pectinase, glucose oxidase, galactose oxidase, lactase, and mixtures thereof."

The present inventors market a product containing lactobacillus and an enzyme system . The composition was a trade secret. The ratio of digestive enzyme units to colony forming units is estimated to be 6.8 digestive enzyme units to $10^7$ colony forming units based upon the input ingredients. The amount of enzyme per feeding was $2.7 \times 10^3$ digestive units per oz. (28.3 g).

In addition to mad cow disease, it is postulated that many other diseases are transmitted to cattle by feeding animal protein and other animal derived products such as poultry manure to the cattle. The statistics on food poisoning caused by infected meat in the United States are appalling, in spite of all the U.S. Government regulations.

On page 141 Tierno states, "According to the federal Government's General Accounting Office, the majority of companies in the U.S. cattle industry, perhaps seventy percent of the total, do not comply with the existing rules."

The U.S. Government is attempting to control the spread of "wasting disease"(Mad Cow?) in the wild. One such system is the establishment of "elimination zones".

Another serious problem, which is well known, is water pollution caused by cattle manure and urine. The source of much of the pollution is undigested, water-soluble nutrients that pass through the cattle. In the following claims, water soluble nitrogen compounds exclude nitrogen compounds in which the nitrogen is fixed in a polymer or in a peptide.

## Disclosure of the Invention

The present invention is directed to cattle feed additives formulated to replace disease carrying animal protein additives that have been used in the past. The additive of the present invention eliminates the transmission of disease caused by animal protein additives, is equivalent to animal protein in the digestion of food by cattle and reduces the amount of water soluble undigested waste which passes through the cattle, thus reducing ground and water pollution. The primary advantage of the present invention is increasing ruminal microbial efficiency thus eliminating the need for dangerous bypass protein, which is very expensive, and improving the efficiency on protein utilization. Without being bound by theory, it is postulated that the enzymes of the present invention cause a rapid break down of material, including fiber, in the rumen to provide food for the bacteria, causing a rapid growth of bacteria which are high quality microbial protein. This was a totally unexpected result. As this protein is cheaper to produce than a comparable grade of animal protein is to buy, it creates an incentive to feed cattle the cattle feed additive of the present invention instead of diseased bypass protein.

The present invention is a cattle feed additive containing fibrolytic enzymes having enzyme activity and one or more species of lactobacillus bacteria having colony forming units wherein the ratio of enzyme activity to colony forming units of at least about 1 unit of digestive enzyme activity to every $10^5$ colony forming units. Preferably the cattle feed additive has a ratio of enzyme activity to colony forming units of at least 2 units of enzyme activity to every $10^6$ colony forming units. Preferably the lactobacillus bacteria are selected from the group comprising *Lactobacillus Acidophilus, Lactobacillus Plantarum,* and

*Lactobacillus Brevis*, and mixtures thereof. Preferably the fibrolytic enzymes are selected from the group comprising cellulases, xylanase, hemi-cellulase and mixtures thereof.

The composition of the present invention can be free of surfactants and any other ingredients disclosed in the prior art to enhance the performance of enzymes and/or lactobacillus bacteria.

The method of making cattle feed of the present invention is characterized by replacing previously used bypass protein in the animal feed with a sufficient amount of a mixture of one or more specie of lactobacillus bacteria and one or more types of fibrolytic enzymes, to produce at least enough microbial protein to be at least equivalent to one half pound (.23kg) of animal protein fed to each of the cattle per day, assuming that each of the cattle are mature and of an average weight for cattle. The preferred lactobacillus bacteria are selected from the group consisting of *Lactobacillus Acidophilus, Lactobacillus Plantarum,* and *Lactobacillus Brevis*, and mixtures thereof, and the protein byproducts replaced are selected from the group consisting of nerve, brain, blood, bone and meat containing byproducts. The preferred lactobacillus bacteria are a mixture of *Lactobacillus Acidophilus, Lactobacillus Plantarum,* and *Lactobacillus Brevis.* The one or more digesting enzymes are preferably selected from the group consisting of xylanase, and cellulases derived from *Trichoderma viride, Aspergillus oryzae, Aspergillus niger,* and *Bacillus subtilis.* Preferably the one or more digesting enzymes are a mixture of xylanase, and cellulases derived from *Trichoderma viride, Aspergillus oryzae, Aspergillus Niger,* and *Bacillus subtilis.*

The method of converting cattle feed to microbial protein in cattle of the present invention is also characterized by incorporating a sufficient amount of a mixture of one or more species of lactobacillus bacteria and one or more types of digesting enzymes into cattle feed to form at least a sufficient amount of microbial protein to be at least equivalent to one fourth pound (.11 kg) of animal protein fed to each of the cattle per day. The lactobacillus bacteria are preferably selected from the group consisting of *Lactobacillus Acidophilus, Lactobacillus Plantarum,* and *Lactobacillus Brevis*, and mixtures thereof and the amount of

microbial protein formed is at least equivalent to one half pound (.23kg) of animal protein fed to each of the cattle per day. The lactobacillus bacteria are preferably a mixture of *Lactobacillus Acidophilus, Lactobacillus Plantarum,* and *Lactobacillus Brevis.* The one or more digesting enzymes are preferably selected from the group consisting of xylanase, and cellulases derived from *Trichoderma viride, Aspergillus oryzae, Aspergillus niger,* and *Bacillus subtilis.* One or more digesting enzymes are preferably a mixture of xylanase, and cellulases derived from *Trichoderma viride, Aspergillus oryzae, Aspergillus Niger,* and *Bacillus subtilis.*

In another embodiment the cattle feed of the present invention is characterized by the daily ration of feed fed to each head of cattle containing a sufficient amount of one or more strains of lactobacillus bacteria and one or more types of digesting enzymes having an enzyme activity of at least $10^4$ digestive units per oz (28.35 g) to digest the cattle feed for the bacteria and increase the number of bacteria in the rumen which are microbial protein. Preferably the enzymes are present at a level sufficient to produce an enzyme activity of from $10^4$ to $10^8$ units per gram of cattle feed and the lactobacillus bacteria being present at a level sufficient to increase the yield of microbial protein in the rumen. The microbial protein is produced in the cattle by interaction of the bacteria and enzymes, the bacteria are preferably present at a level of from $10^6$ to $10^{10}$ colony forming units per gram of cattle feed and enzymes are preferably present at a level sufficient to produce a digestive enzyme activity of from $10^6$ to $10^7$ units per gram of cattle feed.

The method of the present invention reduces runoff of water soluble nitrogen compounds from cattle manure comprises incorporating a sufficient amount of a mixture of one or more species of lactobacillus bacteria and one or more types of digesting enzymes into cattle feed to form at least a sufficient amount of microbial protein to be at least equivalent to one fourth pound (.11 kg) of animal protein fed to each of the cattle per day. This provides a

better amino acid balance and the production of insoluble nitrogen compounds. The cattle feed of the present invention can be free of a surfactant on a carrier.

## Modes for Carrying Out the Invention

The Protein Edge™ (PE) feed additive of the present invention contained the following ingredients. The active ingredients portion contained 8.8 pounds (4 kg) of Ruminant Formula 40 AF and 80 pounds (36.3 Kg) of M8C enzymes.

Ruminant Formula 40 AF contains a mixture of *Lactobacillus Acidophilus, Lactobacillus Plantarum* , and *Lactobacillus Brevis*. These are live, concentrated bacteria suspended in a mixed sugar base. The bacteria are in a weight % ratio of *Lactobacillus Acidophilus* 60%, *Lactobacillus Plantarum* 20%, and *Lactobacillus Brevis* 20%. .The final concentration with the sugar base is blended to 80 billion cfu/gram with a guarantee of 40 billion cfu/gram. The bacteria were prepared according to the procedure of U.S. Patent 4,226,940 Storrs (1980).

M8C enzymes are a dried fermentation extract of *Bacillus subtilis, Aspergillus oryzae, Trichoderma viride* and *Aspergillus niger*. M8C enzymes are a 50/50 mixture by weight of EX 28000 enzymes and Multicel 185 enzymes.

Enzymes that can be used in the practice of the present invention are available from NOVOZYMES JEFFREYS BIOLOGICALS, Inc. Salem, VA. in products known as Xylanase, Maxicel™, Multicel™ and EX 28000™ enzymes.

*Xylanase* acts on D-Xylan in a manner reminiscent of *alpha* and *beta amylase* on starch and results in the production of D-Xylose. Due to interactions with pectin and hemicellulose, *Xylanse* has a considerable amount of *pectinase* added. The activity is 30,000 to 150,000 *Xylanase* Units/g. This product also contains high levels of *cellulase*, *pentosanase* and *pectinase*.

Many *cellulases* of fungal orgin are known for their activity range extending well into the lower pH values. Maxicel and Multicel are two such companion products with very concentrated cellulolytic activity.

Multicel™ 185 cellulase is a combination of cellulases from *Aspergillus oryzae, Trichoderma viride* and *Aspergillus niger*. Multicell 185 has a cellulase activity, at pH 6.5, of 185,000 units/g. Assay Method: $C_1$ -ase as well as CMC -ase. Multicel also has some xylanase activity.

EX 28000 enzymes product is a water dispersible blend of the extracts of *Bacillus subtilis* and *Aspergillus oryzae*. The product includes high concentrations of alpha-amylase, beta-glucanase (gumase), and hemi-cellulase. The product has an Amylolytic Activity of 28,000 BAU/gram, a Betaglucanase Activity of 12,000 Betaglucanase units/gram and a Hemicellulase Activity of 900 Hemicellulase units/gram. Although a primary enzyme associated with *Bacillus subtilis* extract is amylase, other useful hydrolases are often included in this product. These other enzymes catalyze the breakdown of complex carbohydrates other than starch. Hemi-cellulase activity attacks plant wall components. Beta-glucanase helps break down beta-linked glucose polymers often associated with grains, such as barley, oats, and wheat, and other products, including soy bean meal and locust bean gum. This additional digestive action is broadly classified as gumase activity. The presence of soluble calcium has a stabilizing effect on most enzymes of this type.

Based upon the above ingredients the feed additive contained $1.6 \times 10^{14}$ colony forming units per 2000 (907.2 Kg) pounds of feed additive and an enzyme activity of $4.09 \times 10^{9}$ per 2000 pounds (907.2 Kg) giving a ratio of enzyme activity to colony forming units of 1 to $1.16 \times 10^{5}$.

Other ingredients in the feed additive of the present invention include 987.2 pounds (447.8 kg) of calcium carbonate, 400 pounds (181.4 kg) of corn gluten, 500 pounds (226.8 kg) of dried molasses and 24 pounds (10.9 kg) of mineral oil. The total weight of the feed

additive was 2,000 pounds (907.2 Kg). Each ounce (28.35 g) of the feed additive contained 3 X $10^9$ colony forming units and 1.25 X $10^5$ units of enzyme activity. The numbers are approximate because of the instability of the colony forming units, and the presence of enzyme activity in addition to the enumerated enzyme activity.

Example 1

A total mixed ration (TMR) balanced for 70 pounds (31.6 kg) per day of milk production was prepared as shown in TABLE 1. Based upon an assumed intake of 50 pounds (22.7 kg) a day, the Protein Edge (PE) feed additive of the present invention was added to four batches of the TMR at levels of 0, .75, 1.0 and 1.5 ounces (0, 21.3, 28.4, 42.5 gms) per 50 pounds (22.7 kg) of TMR.